

NF 187/01

We claim:

1. A novel oligonucleotide primer for phosphatidyl inositol in *B. cereus* said primer comprising
 - PI - 1 (F) 5' AGTATGGGGAATGAG 3'
 - PI - 2 (R) 5' ACAATTTTCCCACGA 3'
2. A method for the detection of *B. cereus* in foods said method comprising using primers specific for phosphatidyl inositol gene in *B. cereus* in a mixed microflora, said primers comprising
 - PI - 1 (F) 5' AGTATGGGGAATGAG 3'
 - PI - 2 (R) 5' ACAATTTTCCCACGA 3'
3. A method as claimed in claim 2 wherein the food matrices for detecting *B. cereus* are milk and cooked rice.
4. A method as claimed in claim 2 wherein template DNA from *B. cereus* in cooked rice is extracted using Triton X-100, 0.5 - 2%, boiling at 96 - 100°C for 3 - 8 min and treatment with phenol : chloroform in the ratio of 22 : 21 - 28 : 27.
5. A method as claimed in claim 2 wherein the template DNA from *B. cereus* in milk is extracted using diethyl ether : chloroform in the ratio of 1:1 - 1 : 3, urea 1.5 - 3.5 M and sodium dodecyl sulphate in a range of 0.5 - 2%.
6. A method as claimed in claim 2 wherein the PCR reaction mixture in a total volume of 25 µl comprises of Tris HCl: 8 - 12 mM; KCl: 45 - 55 mM; MgCl₂: 0.5 - 3.0 mM; gelatin: 0.005 - 0.02%; individual deoxynucleoside triphosphates: 150 - 300 µM; each specific primer: 30 - 60 picomoles; Taq DNA polymerase: 0.5 - 2.0 units and template DNA: 1 - 3 µl.
7. A method as claimed in claim 2 wherein detection of *B. cereus* is effected by amplification profile of target gene from an initial denaturation at 90 - 98°C for 2 - 8 min, amplification cycles of 28 - 40, each cycle with a denaturation at 90 - 98°C for 40 - 70 seconds, annealing at 46 - 54°C for 40 - 80 seconds and an extension at 68 - 76°C for 45 - 75 seconds and final extension at 68 - 76°C for 4 - 12 min.
8. A method as claimed in claim 2 wherein analysis of the PCR product is done in 1.2 - 1.8% agarose gel electrophoresis, visualization of the PCR product by staining with 0.5 (g/ml ethidium bromide and observation in a UV transilluminator.
9. A method as claimed in claim 2 wherein detection of minimum number of cells of *B. cereus* is done in a food matrix by PCR.